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A new species of *Araneus* (Araneae: Araneidae) from Japan

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Abstract — A new species of *Araneus* is described under the name *Araneus reizan* n. sp., by using specimens collected from Honshu, Japan. The female of the species can be distinguished from related species by the epigynal opening directing inward. The male of the species closely resemble *A. macacus*, but can be separated from the latter by the wider conductor, thinner projection of the conductor, and larger projection of the tegulum. The pairing of male and female specimens of the species was confirmed by DNA barcoding using mt-COI partial sequencing data.

Key words — *Araneus reizan*, barcoding, COI, taxonomy

Introduction

The genus *Araneus* Clerck 1757 is a large genus comprising around 600 species that have been described worldwide (World Spider Catalogue 2019), and 37 of these species have been known in Japan (Tanikawa 2019). Species of this genus are well studied; they can be easily found due to their relatively large body size and orb-webs. However, several interesting specimens of unknown species were obtained during a continuous survey of spider fauna in Japan. From this survey, a new species was identified and is described in this paper.

Materials and Methods

The specimens were preserved in a 75% ethanol solution at room temperature (18–27°C). The morphological characteristics were examined under a stereoscopic microscope (M3Z, Wild Heerbrugg AG, Heerbrugg, Switzerland), and photographs were taken by using an EOS Kiss X7 with an EF-S 60-mm f2.8 macro photo lens and a MT-24EX macro twin flash (Canon Inc., Tokyo, Japan) or attached to the microscope. All measurements are given in millimeters (mm). The type specimens designated in this paper are deposited in the collection of the National Museum of Nature and Science (NSMT), Tsukuba, Japan.

Because the specimens were collected independently in different years and from different localities, I conducted DNA barcoding using mt-COI sequencing data to confirm the conspecific pair of female and male specimens. The specimens used in molecular analysis are shown in the Appendix. Genomic DNA was extracted from the right fourth leg by using a FavorPrep Tissue Genomic DNA Extraction Mini Kit (Favorgen Biotech Corp, Ping-Tung, Taiwan). Mt-COI was amplified by using the primer set LCO1498: 5’-GGT CAA CAA ATC ATA AAG ATA TTG G-3’ and HCO2198: 5’-TAA ACT TCA GGG TGA CCA AAA AAT CA-3’ (Folmer et al. 1994). The reactants were initially denatured for 2 min at 94 °C, followed by 40 cycles of 15 s at 94 °C, 20 s at 47 °C, and 30 s at 72 °C; in some specimens, 20 cycles of 15 s at 94 °C, 20 s at 40 °C and 30 s at 72 °C and 20 cycles of 15 s at 94 °C, 20 s at 45 °C and 30 s at 72 °C. PCR products were purified by using ExoSAP-IT (GE Healthcare Life Sciences, Little Chalfont, United Kingdom). Purified PCR products were sequenced by using a BigDye Terminator Cycle Sequencing kit (ver. 3.1, Applied Biosystems, Foster City, CA, USA) and analyzed on an ABI 3130xl automated DNA sequencer (Applied Biosystems), or sequenced by the Takara Bio CDS Center (Takara Bio, Shiga, Japan) using an ABI 3730xl DNA Analyzer (Life Technologies Japan Ltd., Tokyo, Japan).

Chromatograms were checked by using MEGA ver. 7 (Kumar et al. 2016). Obtained sequences were aligned by using MUSCLE (Edgar 2004) in MEGA. The phylogenetic tree was constructed by the neighbor-joining (NJ) method by using MEGA.

Results

I obtained 603 bp of mt-COI partial sequences from the specimens for molecular analysis. Species names, haplotype names, and their accession numbers are shown in the Appendix. Two females and two males of the species in question formed a monophyletic group in the inferred phylogenetic tree (Fig. 1, haplotype names with ARE). Thus I considered that these females and males belong to the same species.

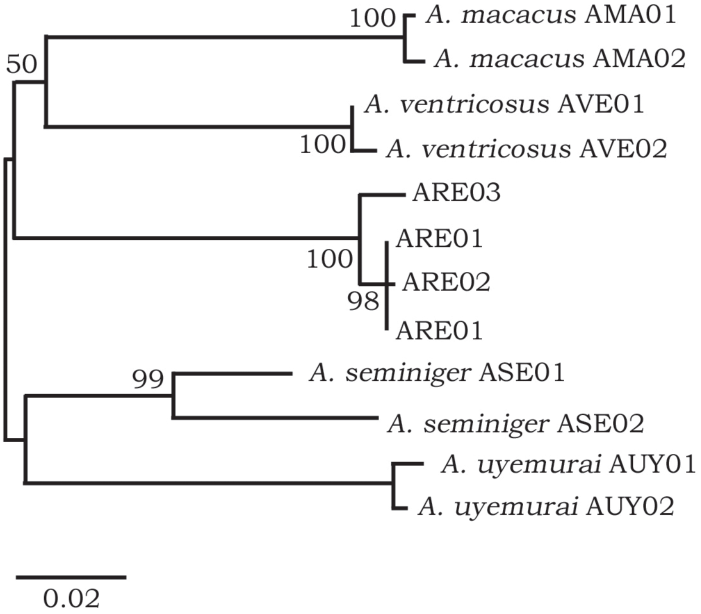


Fig. 1. An unrooted tree obtained by the neighbor-joining method for the detected haplotypes. Scores at each node are bootstrap values (1000 replicates). Specimens of haplotypes ARE01–03 are described as *A. reizan* n. sp. in this paper. The scale bar shows substitutions per site.

The remaining specimens, which were not used for DNA analysis, were identified morphologically.

The species in question resembles *Araneus ventricosus* (L. Koch 1878), *A. macacus* Uyemura 1961, *A. uyemurai* Yaginuma 1960, and *A. seminiger* (L. Koch 1878), but it can be separated from these by the morphology of epigyne and male palp (see the Diagnosis). Consequently, I came to the conclusion that the species in question is new to science.

Description of the new species

Araneus reizan new species

[Japanese name: Yamabushi-onigumo]
(Figs. 2–11)

Type series. Holotype: ♀, Mt. Seppiko, Himeji-shi, Hyogo Prefecture, Japan, 16-VIII-2015, K. Yamamoto leg. (COI haplotype: ARE01). Paratypes: 1♀, Ekari, Kuzumaki-machi, Iwate Prefecture, 15-VIII-2000, S. Okawa leg; 1♂, Mt. Tatsugane, Minami-sanriku-cho, Miyagi Prefecture, 20-VII-2013, A. Shinkai leg. (COI haplotype: ARE02); 1♀, Mt. Tsukuba, Tsukuba-shi, Ibaraki Prefecture, 5-VIII-2019, Y. Suzuki leg. (COI haplotype: ARE03); 1♂, Kamogawa-cho, Toyota-shi, Aichi Prefecture, K. Ogata leg. (COI haplotype: ARE01); 1♂, Nenoue Kogen, Nakatsugawa-shi, Gifu Prefecture, 21-VII-2007, K. Ogata leg.

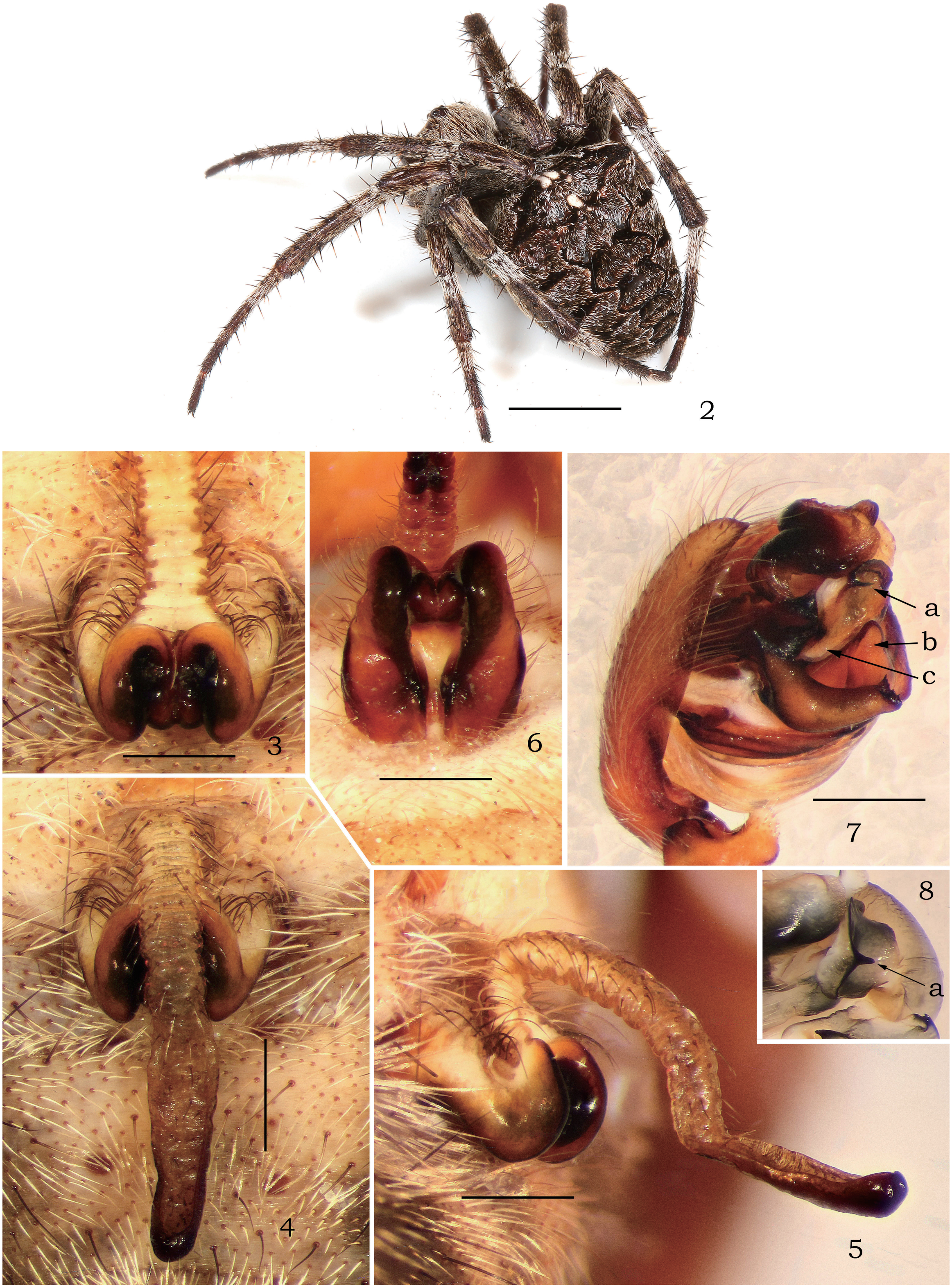
Etymology. The specific name is derived from its type locality. The Japanese word "reizan" means the holy mountain. The type locality of the present new species, Mt. Seppiko, is famous as a holy mountain for "Shugendo" or Japanese mountain asceticism-shamanism incorporating Shinto and Buddhist concepts.

Diagnosis. Females of the new species can be separated from those of the related species, *A. ventricosus*, *A. macacus*, *A. uyemurai*, and *A. seminiger* by the epigynal opening directing inwardly to the center of epigyne (Figs. 9–10, arrow). The shape of male palp of the new species closely resembles that of *A. macacus*, but can be distinguished from the latter by the following points, the conductor is wider (Figs. 7, 11, arrow c), the projection of the conductor is thinner (Figs. 7–8, 11, arrow a), and the projection of the tegulum near the conductor is larger (Figs. 7, 11, arrow b).

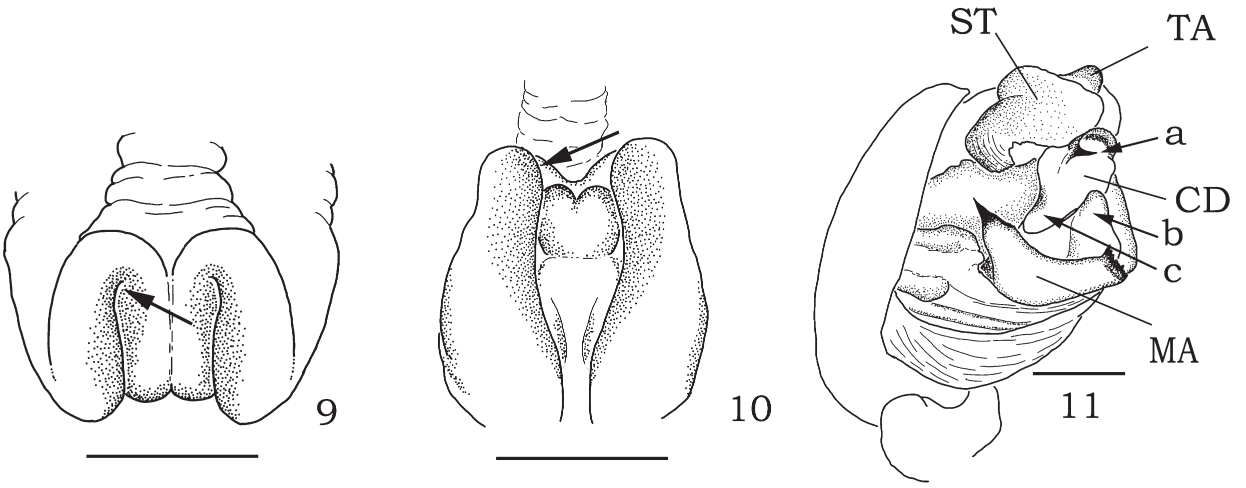
Description. Based on the holotype female and one male paratype from Miyagi.

Coloration and markings. Female (Fig. 2) and male: carapace reddish dark brown. Dorsum of abdomen dark brown with black folium and fine patterns by black and light color.

Measurements. Female / male. Body 15.23 / 10.62 long. Carapace 7.70 / 5.75 long; 5.80 / 4.38 wide. Length of legs [tarsus + metatarsus + tibia + patella + femur = total]: I, 2.00 + 5.60 + 5.50 + 3.30 + 6.60 = 23.00 / 1.60 + 5.20 + 6.10 + 2.80 + 6.70 = 22.40; II, 2.10 + 6.20 + 6.10 + 3.30 + 7.40 = 25.10 / 1.50 + 4.20 + 5.40 + 2.60 + 6.10 = 19.80; III, 1.30 +



Figs. 2–7. *Araneus reizan* n. sp. 2, female (paratype from Ibaraki), dorsal view; 3–4, epigyne, ventral view (holotype); 5, same, lateral view (holotype); 6, same, posterior view (paratype from Iwate); 7, male left palp, prolateral view (paratype from Miyagi). 8. *Araneus macacus*, male left palpal conductor (specimen from Kanagawa). Scales: 2, 5 mm; others, 0.5 mm.



Figs. 9–11. *Araneus reizan* n. sp. 9, epigyne, ventral view (holotype); 10, same, posterior view (paratype from Iwate); 11, male left palp, prolateral view (paratype from Miyagi). CD: conductor, MA: median apophysis, ST: subterminal apophysis, TA: terminal apophysis. Scales: 0.5 mm.

2.90 + 2.90 + 2.30 + 4.70 = 14.10 / 1.10 + 2.60 + 2.90 + 1.70 + 4.60 = 12.90; IV, 1.50 + 4.90 + 4.90 + 2.90 + 7.00 = 21.20 / 1.30 + 4.30 + 4.40 + 2.00 + 6.10 = 18.10. Abdomen 11.10 / 5.88 long; 10.00 / 4.19 wide.

Body and legs. Female / male. Carapace longer than wide [length divided by width 1.33 / 1.31]. Median ocular area wider than long [length divided by width 0.97 / 0.96]; wider in front than behind [anterior width divided by posterior width 1.15 / 1.22]. Labium wider than long [length divided by width 0.80 / 0.78]. Sternum longer than wide [length divided by width 1.12 / 1.39]. Length of leg I divided by length of carapace 2.99 / 3.90. Abdomen longer than wide [length divided by width 1.11 / 1.40].

Genital organs. Epigyne as in Figs. 3–6, 9–10, scape wrinkled, slim and long. Male palp as in Figs. 7, 11, subterminal apophysis distally rounded, tip of embolus hidden by conductor, tegulum with a projection near base of conductor.

Variations. Female body length 14.61–16.46; male body length 9.50–10.62.

Distribution. Japan (Honshu).

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Appendix. Locality, haplotype and accession numbers of specimens used for the molecular analysis.

species	sex	locality	haplotype	accession no.
A. reizan	female	Mt. Seppiko, Himeji-shi, Hyogo Prefecture	ARE01	LC516591
A. reizan	male	Kamogawa-cho, Toyota-shi, Aichi Prefecture	ARE01	
A. reizan	male	Mt. Tatsugane, Minami-sanriku-cho, Miyagi Prefecture	ARE02	LC516592
A. reizan	female	Mt. Tsukuba, Tsukuba-shi, Ibaraki Prefecture	ARE03	LC516593
A. ventricosus	female	Iwatomi, Sakura-shi, Chiba Prefecture	AVE01	LC516594
A. ventricosus	female	Lake Shikotsuko, Chitose-shi, Hokkaido	AVE02	LC516597
A. macacus	female	Tsukuba-shi, Ibaraki Prefecture	AMA01	LC516596
A. macacus	female	Lake Shikotsuko, Chitose-shi, Hokkaido	AMA02	LC516597
A. uyemurai	female	Lake Shikotsuko, Chitose-shi, Hokkaido	AUY01	LC516598
A. uyemurai	female	Yuza-machi, Akumi-gun, Yamagata Prefecture	AUY02	LC516599
A. seminiger	female	Kimitsu-shi, Chiba Prefecture	ASE01	LC516600
A. seminiger	female	Amami-Oshima Island, Kagoshima Prefecture	ASE02	LC516601